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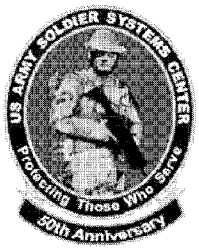

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DEVELOPMENT OF FERMENTED TARO AS A FOOD PRESERVATIVE INGREDIENT IN INTERMEDIATE MOISTURE PRODUCTS

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14. ABSTRACT
This study focuses on the functionality of fermented taro as an antibacterial ingredient for intermediate moisture (IM) products being developed by the military. The taro is cooked and then inoculated with a food-grade bacterium, Lactococcus lactis ssp. lactis, which produces a bacteriocin, nisin, forming a fermented taro product. The fermented taro has antibacterial activity against various bacteria and is freeze-dried for eventual incorporation as a food preservative ingredient in an IM product. L. lactis yielded nisin concentrations in a range of 15,000-19,000 AU/g of taro. Challenge studies were conducted in which the fermented taro wasincorporated into an IM product, the burrito sandwich. The challenge organisms consisted of three starins of Staphylococcus aureus. The burrito samples with 600 AU/g of fermented taro showed no increase in bacterial counts after 7 days However,after 14 days the bacterial counts increased to 3 X 107 CFU/g. The burrito samples treated with 1200 AU/g of fermented taroshowed no increase in growth from the original inoculum (2 X 105 CFU/g) during the challenge study. The last sampling time was at 56 days with a slight decrease in the S. aureus counts. It appears that fermented taro can be a good food preservation ingredient in IM productts, though further studies will have to be done to optimize product.

15. SUBJECT TERMS

POI	BACTERIA	BACTERIOCINS	ANTIBACTERIAL	NATURAL RESOURCES
TARO	MOISTURE	MICROBIOLOGY	TROPICAL REGIONS	STAPHYLOCOCCUS AUREUS
NISIN	SHELF LIFE	FOOD SPOILAGE	FUNCTIONAL FOODS	
RATIONS	FOOD SAFETY	FERMENTATION	FOOD PRESERVATION	

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Preface

This is a report on one part of an integrated project performed under the Biosystems Technology Program (BTP), program element number PE 63001. The study was done from October 2002 to September 2004. Previously this program was funded from August 2000 to September 2001 and subsequently renewed based on the encouraging results obtained during this time frame. The BTP is a congressionally funded program that focuses on environmentally preferable and responsible products and services derived from tropical plants and microorganisms. The funded project is entitled "Development of Taro/Poi into Military/Commercial Functional Foods". Previously a technical report was published on the earlier work conducted from August 2000 to 2001, TR Natick/TR-02/018 entitled "The Antibacterial Potential of Fermented Taro and its Development as a Food Preservative". This report covers the second part of the microbiology and food safety aspects of the project, the development of fermented taro as a food preservative and its incorporation into a developmental military food product, the burrito pocket sandwich. The project involved several team members, including two microbiologists, two food scientists and a chemical engineer at the lead federal agency, Natick Soldier Center, Natick, MA. The goal was to develop the fermented taro as an effective food preservative.

Based on the research, a U. S. Patent has been approved entitled "Method for Making a Food Preservative and for Preserving Food," U. S. Patent No. 10/105,126.

SUMMARY

Congressional funds were appropriated to revitalize the economy in Hawaii by developing products unique to the state. This program looked at taro, a native crop of Hawaii, which has many interesting properties. The funded program evaluates the functional properties of taro and its fermented product poi, its application into existing products and its development into unique military/commercial food items. This final report covers one aspect of the program: the antibacterial potential of the fermented taro, its development as a potential food preservative and its incorporation into a developmental military ration (burrito pocket sandwich).

In Hawaii the fermented taro is called poi. However, in Hawaii it is the native flora present on the taro that produces the fermented product poi. In this study a controlled fermentation of taro is done where a particular bacterium is used in the fermentation. The taro is sterilized and inoculated with a food-grade bacterium capable of producing a small peptide, a bacteriocin. The bacteriocin has antibacterial properties against many bacteria and thus when produced in a food such as taro, it can act as a food preservative. The fermentation bacterium used in this study is *Lactococcus lactis* ssp. *lactis* a nisin producer, a specific bacteriocin. A functional food ingredient is thus developed from the fermented taro for potential applications to military long-term storage food products.

Such a long-term shelf stable military product being developed is the pocket sandwich. The pocket sandwiches are being developed for the military to enhance the variety of individual ration components available, as well as a component that can be eaten on the move. Hurdle technology is used to provide sandwiches with a minimum 3-year shelf life when held at or below 80° F (6 months at 100° F). This technology uses a number of hurdles (preservation techniques) to inhibit the growth of microorganisms. In this study the fermented taro was being developed as another hurdle that could be incorporated into the pocket sandwich. The fermented taro was added to a new intermediate moisture (IM) product under development, the burrito pocket sandwich. This product is usually safe from microbial growth. However, there was a desire to increase the pH and water activity (hurdles) of the product to improve the sensory characteristics of the product. By doing this, the product becomes susceptible to the growth of bacteria, particularly *Staphylococcus aureus*. The fermented taro has activity against *S. aureus* and would act as the third hurdle to prevent the growth of *S. aureus* at the elevated pH and water activity. This technical report describes our findings.

DEVELOPMENT OF FERMENTED TARO AS A FOOD PRESERVATIVE INGREDIENT IN INTERMEDIATE MOISTURE PRODUCTS

INTRODUCTION

Taro (*Colocasia esculenta)*, a food crop of many tropical countries, is well known throughout the Hawaiian Islands as the principal source of a fermented food called poi. In ancient times, poi comprised a large portion of the Hawaiian diet, and today it is still sold commercially in Hawaii and parts of California. The fermentation of taro to poi is a natural fermentation meaning no starter culture is added to the product. The fermentation is accomplished by the native flora present on the taro.

In this study a controlled fermentation of taro is done where a particular bacterium is used in the fermentation process. The taro is inoculated with a bacterium that is capable of producing a bacteriocin. Bacteriocins are small peptides produced by food grade bacteria, such as lactic acid bacteria, which exhibit antibacterial properties against mainly Gram-positive bacteria. The fermentation bacterium used in this study is *Lactococcus lactis ssp. lactis*, a nisin producer. Nisin is probably the most widely studied and used bacteriocin in the food industry. A functional food ingredient is developed from the fermented taro for potential applications to military long-term storage food products. The fermentation of taro by *L. lactis* produces a fermented product having the capability of inhibiting food pathogenic bacteria such as *Staphylococcus*.

The study of the antimicrobial aspects of taro was divided into four phases. The four phases of research are:

Phase I, to determine the antimicrobial properties of the natural fermentation product, poi, with particular emphasis on the presence of bacteriocins.
Phase II, to determine the suitability of taro as a growth medium for cultivation of food safe bacteria capable of producing bacteriocins.
Phase III, to determine feasibility of adding the fermented taro products, produced in phase II, to military rations.
Phase IV, to evaluate the efficacy of fermented taro on the growth activities of targeted foodborne spoilage/pathogenic organisms in specific foods.

Phases I and II were covered in Technical Report, Natick/TR-02/018, June 2002 (Muller et.al. 2002). Phases III and IV are covered in this report.

In phases III and IV the fermented taro is applied as an ingredient in the military's new intermediate moisture (IM) food product, the pocket sandwich. With IM products, a hurdle technology is used to control potential growth of pathogens. In this case the pH and water activity (a_w) are closely monitored and controlled. The particular IM pocket sandwich used in this study was one under development - - the burrito pocket sandwich. This product is usually produced at a water activity of 0.86 and pH of 5 - 5.5. In an effort to improve the sensory characteristics of the product, there was a desire to increase the water activity and pH of the product. The food pathogen that would create a health

problem at the elevated levels under consideration is *Staphylococcus aureus.* In challenge studies the fermented taro is added to the burrito as a third protective element, to pH and a_w, to determine if it can maintain the microbiological stability of the burrito pocket sandwich.

MATERIALS & METHODS

Preparation of Poi

In preparation of taro corms (Fig. 1), the corms are first cooked. The corms are steamed or autoclaved for 20 minutes. The outer skin of the corms is then removed using a knife. After the corm is peeled it is cut into approximately 2" x 2" x 2" pieces. The pieces of corm are then put through a meat grinder three times. The fermentation of the prepared poi is usually very rapid. Within the first 24 hours of preparation, the pH drops from 6.3 to a pH of 4.5. Thereafter, the acidity increases gradually until it reaches an average pH of 3.8 by the third day (Moy and Nip, 1983).

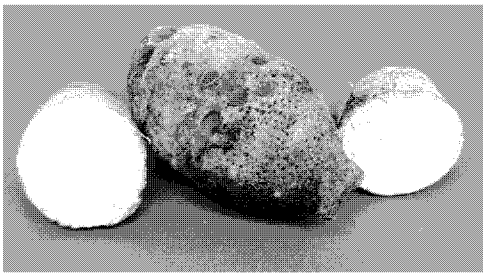

Figure 1. Photo of taro corm.

Bacteriocin Assay

The bacteriocin activity was determined by a well diffusion assay (Tagg and McGiven, 1971). Before the samples are assayed, the viable bacteria in the sample are heat killed by placing the sample in a water bath at 80° C for 20 minutes. Samples are placed in Trypticase Glucose Yeast Extract (TGE) agar wells and to facilitate diffusion of the bacteriocin, the agar plates were stored at 2-5° C for 5-6 hrs after application of the bacteriocin sample. Subsequently, TGE agar plates were overlaid with 10 ml of TGE soft agar (0.75% agar) inoculated with an overnight culture of test organism, *Lactobacillus plantarum* or *Micrococcus luteus,* depending on the bacteriocin being screened. The plates were then incubated at 37° C for 24 hrs. Plates were subsequently examined for zones of inhibition (Fig. 2). Activity is expressed as activity units/gram (AU/g) of material.

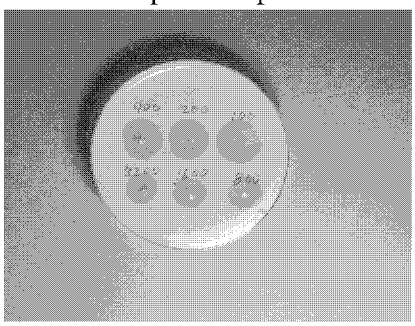

Figure 2. Petri dish showing zones of clearing.

Preparation & Optimization of Nisin Production in Fermented Taro

The taro corm was prepared as stated above under preparation of poi. However, after the taro is ground the material is freeze-dried and stored at freezer temperatures (-20° C). The freeze-dried taro was used to prepare a 2% taro solution (weight/volume) for the fermentation. *Lactococcus lactis ssp lactis* ATCC 11454 was used to inoculate the taro

solution. To the 2% taro solution a small amount of yeast extract was used to supplement the fermentation. The concentration of yeast extract added was 0.01 % (w/v). The addition of yeast extract significantly enhanced the production of active nisin. Food grade yeast extracts used in the study were Provesta's Ohly KAT and Ohly STT (Provesta Food Ingredients, Hutchinson, MN). The addition of yeast extract shortened the time for the drop of the pH and enhanced the nisin yield. After the fermentation is done, the taro is freeze-dried and was ready for incorporation into a food product.

Formulation for Filling of IM Burrito Pocket Sandwich (Tables 1 & 2)

Table 1. The table represents the formulation for the IM burrito pocket sandwich filling with and without fermented taro used in second challenge study. Two different concentrations of fermented taro were used for the challenge study, 100 and 300 au/g of burrito

Ingredients	Original Burrito Formulation (%)	Control Burrito Formulation w/taro (%)	Formulation w/Taro/ Nisin 100 AU/g Burrito (%)	Formulation w/Taro/Nisin 300 AU/g Burrito (%)
*Meat mix	45.52	43.52	44.52	43.52
Tomato paste	16.96	16.25	16.96	16.25
Dehy. onion	0.78	0.78	0.78	0.78
Chili powder	0.78	0.78	0.78	0.78
Dehy. pepper	0.32	0.32	0.32	0.32
Cumin	0.19	0.19	0.19	0.19
Garlic powder	0.12	0.12	0.12	0.12
Ground red pepper	0.10	0.10	0.10	0.10
Liquid lecithin	0.16	0.16	0.16	0.16
Sodium Bicarbonate	0.00	0.25	0.25	0.25
Taro	**0.00**	**3.75**	**0.0**	**0.0**
Taro/nisin	**0.00**	**0.0**	**1.25**	**3.75**
Cheese	35.00	34.00	35.00	34.00
Total	**100**	**100**	**100**	**100**

* Meat mix prepared by vacuum tumbling 85% ground beef 1:1/2 (beef:brine). Brine solution composed of 77% water, 21% glycerol and 2% salt. The meat mix was cooked in iron skillet until most of the free water was burned off.

Table 2. The table represents the formulation for the IM burrito pocket sandwich filling with and without fermented taro used in third challenge study. Two different concentrations of fermented taro were used for the challenge study, 600 and 1200 AU/g of burrito.

Ingredients	Original Burrito Formulation (%)	Control Burrito Formulation w/taro (%)	Formulation w/Taro/ Nisin 600 AU/g Burrito (%)	Formulation w/Taro/Nisin 1200 AU/g Burrito (%)
*Meat mix	45.52	40.0	40.0	34.0
Tomato paste	16.96	15.0	15.0	14.0
Dehy. onion	0.78	0.78	0.78	0.78
Chili powder	0.78	0.78	0.78	0.78
Dehy. pepper	0.32	0.32	0.32	0.32
Cumin	0.19	0.19	0.19	0.19
Garlic powder	0.12	0.12	0.12	0.12
Ground red pepper	0.10	0.10	0.10	0.10
Liquid lecithin	0.16	0.16	0.16	0.16
Sodium Bicarbonate	0.00	0.25	0.25	0.25
Taro	**0.00**	**12.0**	**0.0**	**0.0**
Taro/nisin	**0.00**	**0.0**	**12.0**	**24.0**
Cheese	35.00	30.3	30.3	25.3
Total	**100**	**100**	**100**	**100**

* Meat mix prepared by vacuum tumbling 85% ground beef 1:1/2 (beef:brine). Brine solution composed of 77% water, 21% glycerol and 2% salt. The meat mix was cooked in iron skillet until most of the free water was burned off.

Bread Formulations (Table 3)

Table 3. Two bread formulations used for burrito pocket sandwiches made at pH 5.0 and pH 6.0.

	Acidic (pH 5.0) %	**Basic (pH 6.0)** %
Bread flour	52.25	52.00
Water	32.20	32.30
Shortening	9.10	9.10
Yeast	2.22	2.22
Salt	1.30	1.30
Sucrose ester	1.00	1.00
Control S	0.55	0.55
Gum arabic	0.55	0.55
Calcium sulfate	0.30	0.30
Xantham gum	0.30	0.30
Sorbic acid, encapsulated	0.15	0.10
Sodium bicarbonate	0.00	0.27
	100.0	100.0

Challenge Studies

Three bacteria were used in the challenge studies, three strains of *Staphylococcus aureus*, *S. aureus* ATCC 6538, *S. aureus* ATCC 27154 and *S. aureus* ATCC 8095. The first challenge study was conducted to determine how well the *S. aureus* strains grow in the burrito pocket sandwich at a pH 5 and pH 6. Table 3 shows the bread formulation at pH 5 and 6. The burrito is inoculated at the bread/filling interface, stored and ground at time of sampling. There was no taro in the samples tested and challenge study went for 49 days.

In second challenge study all burritos were at a pH 6. Four sample groups were tested, a control group containing unfermented taro, a group with 100 AU/g of burrito (fermented taro in filling), a group with 300 AU/g of burrito (fermented taro in filling) and a fourth group with 300 AU/g of burrito (fermented taro in the filling and bread). The burrito is inoculated at the bread/filling interface, stored and ground at time of sampling. The challenge study storage time was for 35 days.

In the third challenge study changes were made in the inoculation and processing of samples. Instead of inoculating samples at the bread/filling interface the burritos were ground after preparation and the ground product was inoculated and stored for different sampling times. This provided a more uniform sample for the inoculant in an attempt to obtain more consistent results. Three sample groups were tested, a control group containing unfermented taro, a second group with 600 AU/g of burrito (fermented taro in

filling), and third group with 1200 AU/g of burrito (fermented taro in filling). All samples were at a pH of 6. Storage test went for total of fifty-six days.

RESULTS

A batch fermentation of taro inoculated with *L.lactis*, a nisin producer, is used to produce the fermented taro having inhibitory activity against *S. aureus* and other bacteria. The relative activity of the fermented taro ranged between 15,000-19,000 AU/g of taro. This was determined by the well diffusion method (Tagg and McGiven, 1971), test organism *M. luteus*. The freeze-dried fermented taro is incorporated into the burrito pocket sandwich filling in the appropriate amount to produce a pocket sandwich with the desired AU/g of burrito.

A challenge study was conducted in which the growth of three strains of *S. aureus* was used as the challenge organism in the burrito pocket sandwich. The three *S. aureus* stains were *S. aureus* ATCC 6538, *S. aureus* ATCC 27154 and *S. aureus* ATCC 8095. The challenge organisms were tested in burritos prepared at the usual pH of 5 and at an elevated pH of 6. The burritos were at a water activity of 0.89. Figure 3 shows the results of the challenge study.

A second challenge study was conducted with fermented taro added to the burrito pocket sandwich, prepared at a pH 6. Four different samples were challenged with the three strains of *S. aureus* previously used in first challenge study. A control sample, taro/nisin 0, were burritos having taro in the filling but no nisin activity. The samples taro/nisin 100 AU/g and 300 AU/g were burritos made with fermented taro at the stated AU/g in which the fermented taro was only in the filling. The concentration of nisin in these samples was calculated based on the total weight of the bread and filling. The fourth sample taro/nisin 300 BF AU/g is burritos made with fermented taro at the stated AU/g in which the fermented taro is in the burrito's filling and bread. The burritos were at a water activity of 0.9. Figure 4 shows the results of the challenge study.

A third challenge study was conducted at higher levels of fermented taro incorporated in the filling of the burrito pocket sandwich, at a pH 6. Three samples were challenged with the three stains of *S. aureus*. In this study the challenge organisms were inoculated into the burrito after it was ground to make a more uniform product. In the previous challenge studies the inoculation was at the bread/filling interface. A control sample taro/nisin 0 was a burrito with taro in the filling but no nisin activity. The samples taro/nisin 600 AU/g and taro/nisin 1200 AU/g were burritos made with fermented taro in the filling at the calculated AU/g of burrito stated. The burritos were at a water activity of 0.92. Figure 5 shows the results of the challenge study.

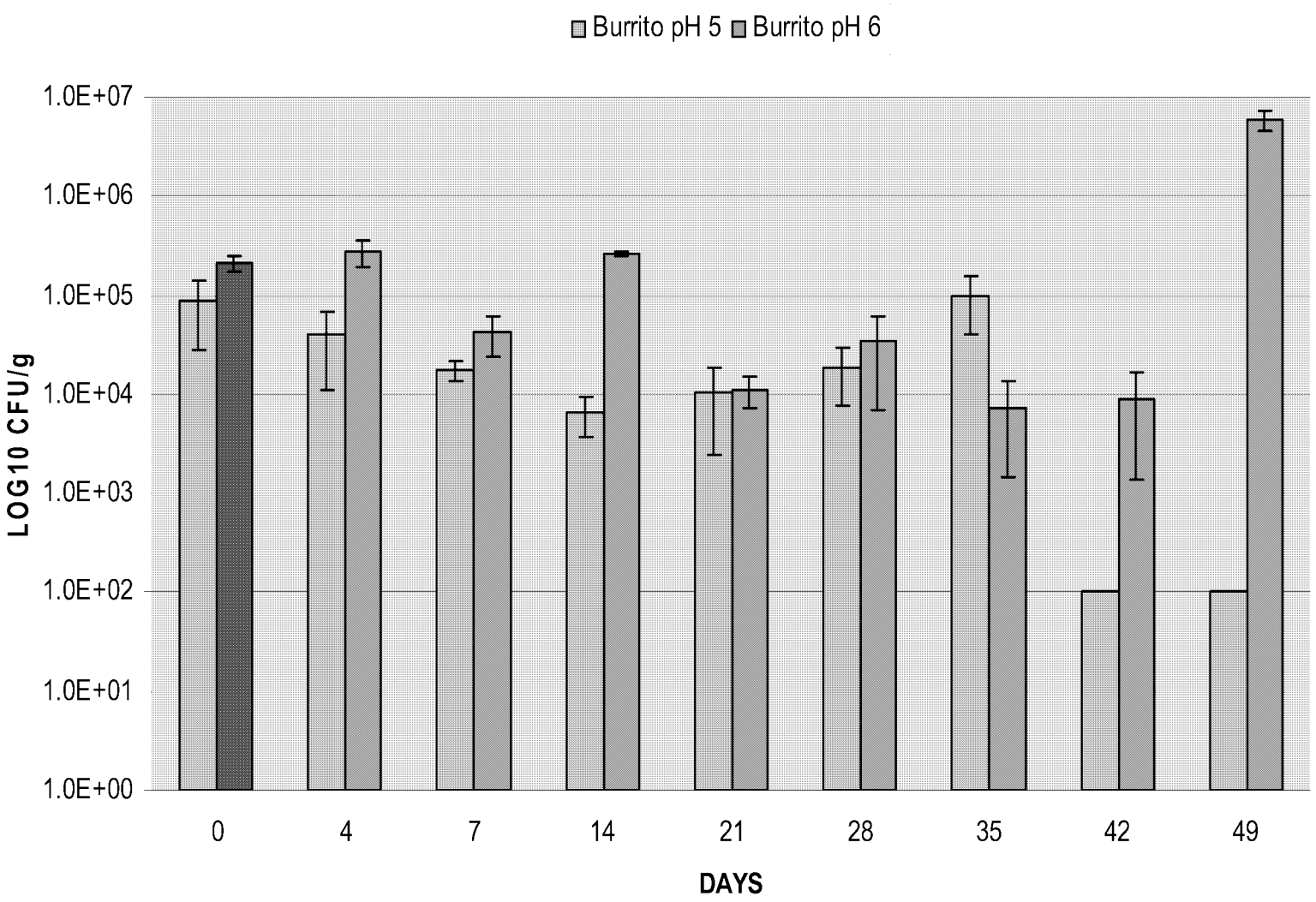

Figure 3. Results of challenge study determining the growth of *S. aureus* in burritos prepared at pH 5, the normal pH of the product, and pH 6. The burritos were challenged with three strains of *S. aureus*.

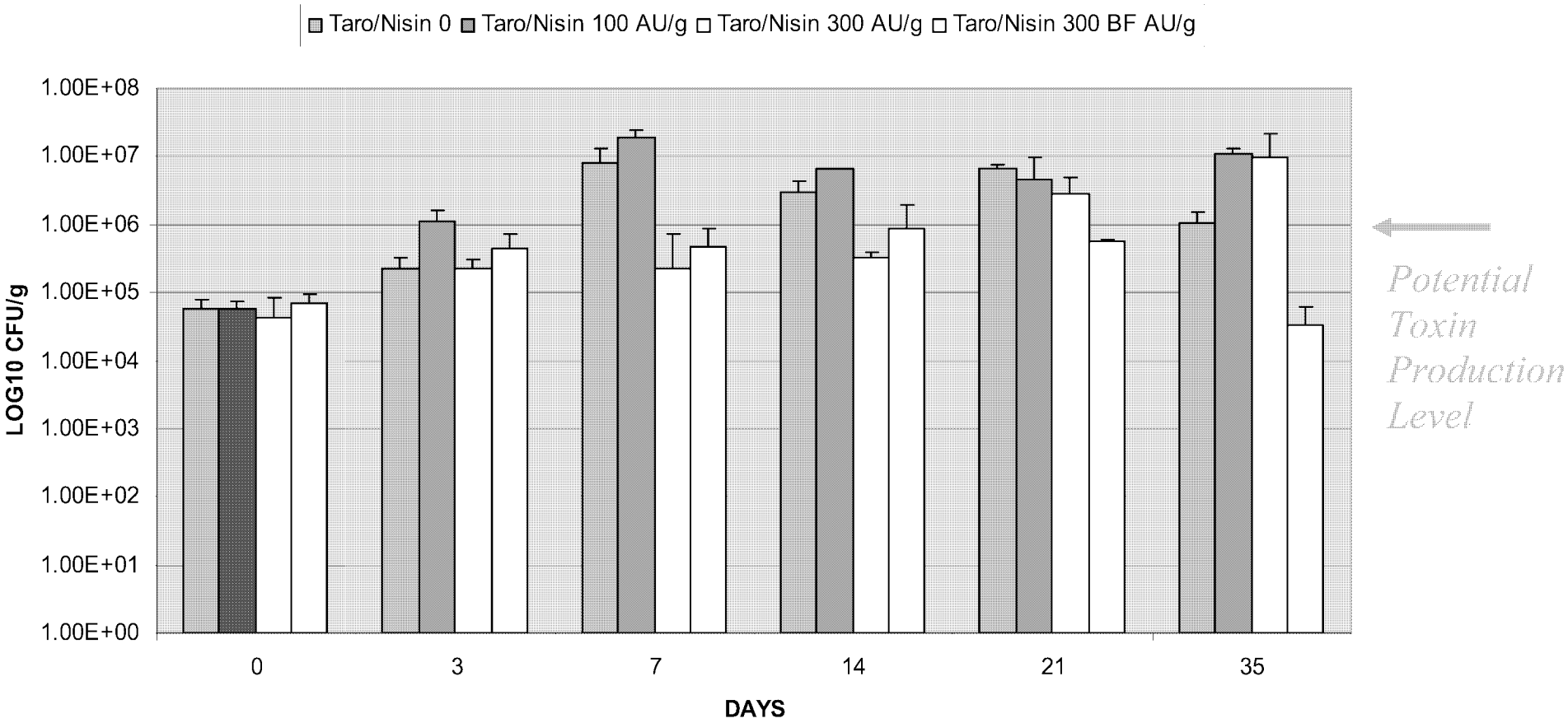

Figure 4. Results of challenge study that determined the growth of three strains of *S. aureus* in the burrito pocket sandwich in which taro and fermented taro are incorporated at different concentrations into the product. There were four burrito sample types, taro/nisin 0 (taro with no nisin activity), taro/nisn 100 AU /g (100 AU/g of burrito, nisin activity in filling), taro/nisin 300 AU/g (300 AU/g of burrito nisin activity in filling) and taro/nisin 300 BF AU/g (300 AU/g of burrito nisin activity in filling and bread). The burritos were at a pH 6.

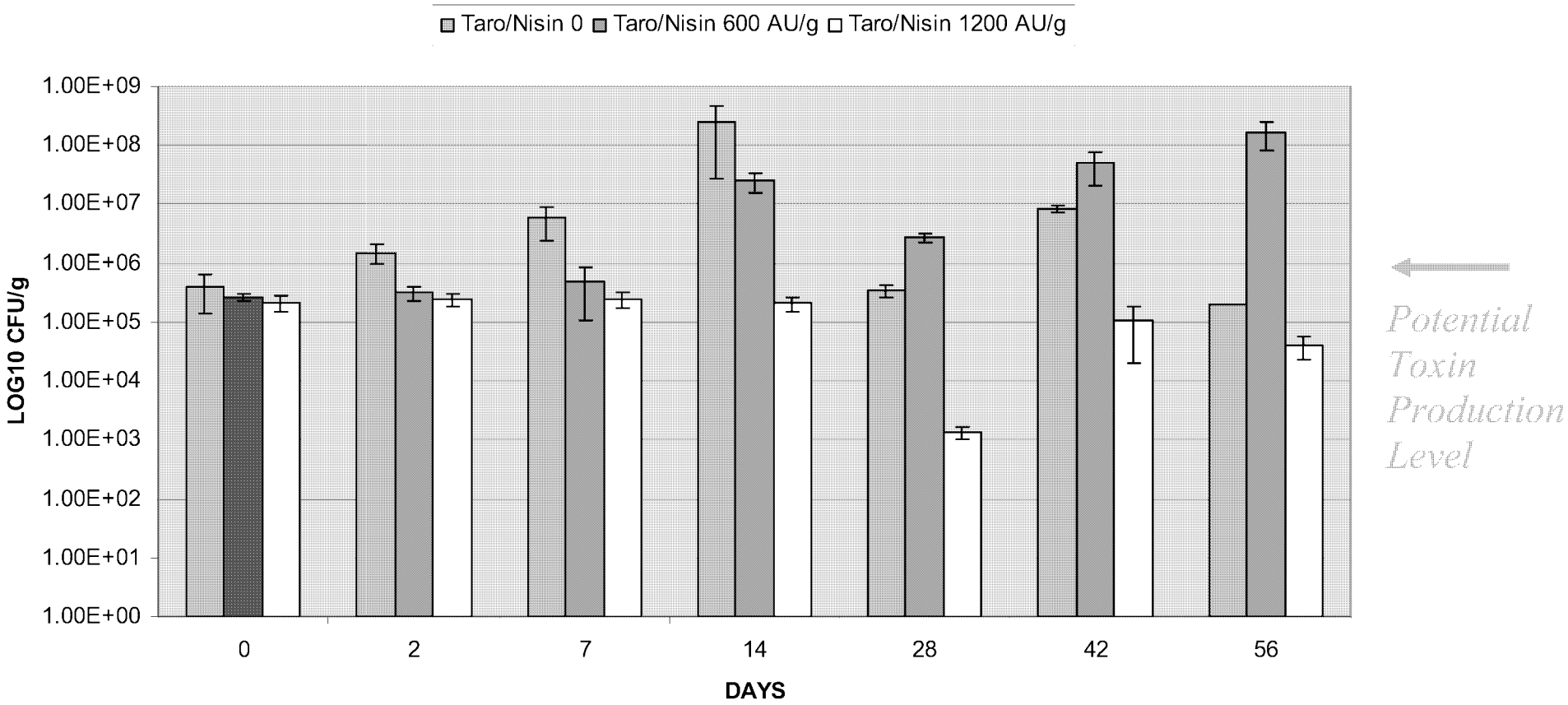

Figure 5. Results of challenge study that determined the growth of three strains of *S. aureus* in burrito pocket sandwich in which taro and fermented taro are incorporated at different concentrations in the product. There were three burrito sample types, taro/nisin 0 (taro with no nisin activity), taro/nisin 600 AU/g (600 AU/g of burrito, nisin activity in filling), and taro/nisin 1200 AU/g (1200 AU/g of burrito, nisin activity in filling). The burritos were at a pH 6.

DISCUSSION

Taro is mainly a starch-based food product. Taro can be naturally fermented to poi by native bacterial flora present on the corm (Allen and Allen, 1933 and Huang et. al., 1994). However, by sterilizing and inoculating the cooked taro with *L. lactis*, a nisin producer, the fermented taro can function as a food preservation ingredient in certain food products. A 2 % taro medium supplemented with a small quantity of yeast extract (0.01 %) can yield active nisin in the range of 15,000 – 19,000 AU/g of taro. Intermediate moisture (IM) products are one type of food product for the application of fermented taro as a food preservative ingredient. The fermented taro has a bland taste to it, thus allowing it to be added to products with little change in sensory characteristics.

Shelf stable pocket sandwiches are being developed for the military to enhance the variety of individual ration components available as well as a component that can be eaten on the move - - an important characteristic of the product. The current military doctrine requires troops to be highly mobile, agile and sustainable under any environmental condition, climate and location. Often times the warfighter does not have the time to stop and prepare a meal. An eat-out-of-hand, eat-on-the-move capability is required for these situations. Hurdle technology is used to provide sandwiches with a minimum 3-year shelf life when held at or below 80° F (6 months at 100° F). This technology uses a number of hurdles (preservation techniques) to inhibit the growth of microorganisms. The use of a combination of several milder barriers rather than a single more severe preservation method produces a safe, stable food with increased quality.

In this study the fermented taro was being developed as another hurdle that could be incorporated into the pocket sandwich. The fermented taro was to be added to a new IM product under development, the burrito pocket sandwich. This product is usually produced at a pH of 5 and a_w of 0.86. At these levels *S. aureus* will not grow in the product. However, there is an interest in the military to increase the pH as well as a_w of the product. This increase would potentially enhance the sensory properties of the burrito. Such an increase in these parameters can also potentially compromise the microbial safety of the product. The fermented taro added to the IM product could provide the necessary protection against *S. aureus*, the microorganism with the greatest potential to compromise the safety of the product, when changes are made in the pH and a_w during long-term storage.

In the first challenge study (Fig. 3) the formulation used for burritos prepared at pH 5 was as listed in table 1 under original burrito formulation. Citric acid was used to drop the pH to 5 if necessary. This same formulation was used for burritos prepared at pH 6, however 0.25 % of sodium bicarbonate was added to the formulation. The water activity of the burritos was at 0.89. No taro or fermented taro was added to sandwiches. This challenge study was done to determine if *S. aureus* would be a problem if the pH and water activity of the product were raised to higher levels then the usual pH 5 and a_w of 0.86. *S. aureus* is not a problem in the product unless the organism grows to levels that allow it to produce significant amounts of their enterotoxins. The *S. aureus* enterotoxins are usually produced when the cell count is above 1 X 10^6 organisms/g of burrito. It is evident from

this study that a pH of 6 is necessary for *S. aureus* growth. The challenge organism in the burrito at pH 5 never reached a level over 1 X 10^5 colony forming units (CFU)/g. In the case of the burrito produced at pH of 6 *S. aureus* growth was close to 1 X 10^7 CFU/g at forty-nine days. This is a good indication that the product's microbial safety has been compromised at this level.

In the second challenge study (Fig. 4), fermented taro was added to the burrito filling at two different levels. The two levels were 100 and 300 AU/g of burrito. Two sets of samples had the fermented taro in only the filling, an additional sample set had 300 AU/g of burrito in both the filling and the bread. The fermented taro was put into the bread and filling to see if it would enhance the effectiveness of the taro. It is believed, that if there is a microbial problem in an IM product, it usually occurs at the bread/filling interface. It is at this interface the challenge organisms are inoculated in the burrito challenge study. Both samples having the 100 and 300 AU/g in the filling, *S. aureus* counts reached 1 X 10^7 CFU/g of burrito. However, the burrito in which the fermented taro was in both the bread and filling the challenge organisms never reached 1 X 10^6 CFU/g. The sample with fermented taro in the bread and filling exhibited the growth of *S. aureus,* however not to the same degree as the other samples in which the fermented taro was only in the filling. This indicates the growth of *S. aureus* was inhibited when the fermented taro was in both the bread and filling, at 300 AU/g of burrito.

In the third challenge study (Fig. 5), the fermented taro was added to the burritos' filling at higher levels than the second challenge study. Two sets of burritos had fermented taro concentrations of 600 AU/g and 1200 AU/g of burrito. In addition, after the burritos were made, each burrito was ground and inoculated with the challenge organisms. Previously, the burrito was inoculated at the bread/filling interface, stored and then ground at time of sampling. The new procedure was used to determine whether it would help to eliminate some of the inconsistent sampling results observed in the second challenge study as well as other challenge studies not reported. It was believed that the uniformity of medium (ground burrito) would provide the necessary environment for the inoculant and sample preparation to produce more consistent sampling results. The control sample and the burritos with fermented taro of 600 AU/g allowed the challenge organisms to grow out to levels above 1 X 10^8 CFU/g of burrito. However, the burrito having fermented taro of 1200 AU/g never reached CFU levels higher then the original inoculum (3 X 10^5/g of burrito).

These results were encouraging. Further studies will have to be done to repeat results obtained, to determine the optimal effective concentration of fermented taro to inhibit *S. aureus* and to determine the effect of the taro on the pocket sandwich during long-term storage.

CONCLUSIONS

- The fermentation of taro by *L. lactis* produced significant amounts of active nisin.
- The yield of nisin is increased significantly when taro is supplemented with a small quantity of yeast extract (0.01%).

- There is no detectable loss of nisin activity during the processing and storage of the fermented taro.
- The fermented taro incorporated into burrito pocket sandwich inhibits the growth of *S. aureus*.
- The fermented taro incorporated into both the filling and the bread produced an enhanced inhibitory effect against *S. aureus*.
- In a fifty-six day challenge study, the fermented taro at 1200 AU/g of burrito prevented the growth of *S. aureus*.
- Intermediate moisture (IM) products are good products for the incorporation of fermented taro as a food preservation ingredient.
- The fermented taro has potential as a functional food ingredient for both military and commercial food products.

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